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Full Paper

Improvement of peripheral vascular impairment by a phosphodiesterase type 5 inhibitor tadalafil prevents oxaliplatin-induced peripheral neuropathy in mice

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ABSTRACT

Oxaliplatin, a platinum-based chemotherapeutic drug, frequently induces peripheral neuropathy. Accumulating evidences suggest a possible relationship between peripheral vascular impairment and peripheral neuropathy. In this study, we investigated the effects of vasodilators on cumulative peripheral neuropathy induced by repeated injections of oxaliplatin (10 mg/kg) once a week for 8 weeks in mice. Single injections of vasodilators, including a phosphodiesterase type 5 inhibitor tadalafil acutely alleviated oxaliplatin-induced cold hypersensitivity, while tadalafil had no effect on the mechanical hypersensitivity. By contrast, long-term administration of tadalafil (0.1% in chow diets) during the oxaliplatin injection period reduced the oxaliplatin-induced decreases in skin temperature and blood flow without affecting platinum concentrations in blood, sciatic nerves, and dorsal root ganglion. The long-term administration significantly suppressed cold, mechanical, and electrical current hypersensitivities as well as thermal hypoesthesia. Furthermore, it prevented the decreases in sensory nerve conductance velocity and the number of endoneurial microvessels, and axon degeneration in the sciatic nerves. *In vitro* studies confirmed that tadalafil does not interfere with the cytotoxicity of oxaliplatin against human cancer cell lines. Altogether, these results suggest that improvement of peripheral vascular impairment by tadalafil could alleviate and prevent oxaliplatin-induced peripheral neuropathy.

1. Introduction

Oxaliplatin (L-OHP) is a platinum-based chemotherapeutic drug widely used to treat colorectal, gastric, and pancreatic cancers. However, it rapidly induces acute peripheral neuropathy in 85–96% of patients, although it is usually improved within a week. Furthermore, cumulative peripheral neuropathy, with paresthesia, dysesthesia, numbness and pain in the hands and/or feet, occurs in 40–93% of patients who have undergone multiple chemotherapy cycles.[1] This cumulative L-OHP-induced peripheral neuropathy (OIPN) diminishes the activities of daily living and quality of life in patients with cancer, often resulting in dosage reduction and delay or even discontinuation of chemotherapy in severe cases.[1–3] Nevertheless, there are no or little effective strategies for preventing or treating OIPN.[3,4]

Endoneurial microvascular dysfunction is observed in patients and animal models with peripheral neuropathy.[5,6] Moreover, peripheral nerve dysfunction in diabetic patients and animal models is preceded by impaired vascular reactivity, which is thus considered an early diagnostic marker and predictor of the severity of diabetic neuropathy.[7–9] Vascular impairment of peripheral arterial diseases is responsible for the induction of sensory disturbance in the early stage, with increasing pain as disease progresses.[10–12] Similarly, experimental animals in which peripheral blood flow in

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the hindlimbs is restricted exhibit spontaneous and evoked pain behaviors as well as tactile hypoesthesia.[13–15] The association between peripheral vascular impairment and peripheral neuropathy is further supported by studies showing that vasodilators, such as phosphodiesterase (PDE) inhibitors,[16–18] prostaglandin analogs,[19] and endothelin (ET) receptor antagonists,[20] improve peripheral vascular impairment but also attenuate neural dysfunction, nerve degeneration, hypoesthesia, and painful behaviors in animal models of diabetes. These findings propose the possibility that improvement of peripheral vascular impairment by vasodilators may be a potential preventive and/or therapeutic strategy for peripheral neuropathy accompanying peripheral vascular impairment.

Peripheral blood flow is reduced also in L-OHP-treated animals.[21,22] Gauchan et al. further reported that persistent improvement of peripheral vascular impairment by a prostaglandin E_1 analog attenuated the mechanical hypersensitivity evoked by a single administration of L-OHP in mice.[21] However, detailed analyses for the efficacy of vasodilators on the cumulative OIPN are still lacking. In the present study, we examined the acute alleviative and preventive effects of vasodilators on the cumulative OIPN in mice. Here, we found that tadalafil, a PDE type 5 inhibitor, has good efficacy against cumulative OIPN. This drug alleviated cold hypersensitivity acutely, but prevented a wide range of symptoms, sensory nerve dysfunction and degeneration observed in cumulative OIPN.

2. Materials and methods

2.1. Details are presented in supplementary materials and methods

2.1.1. Animals

C57BL/6J mice (Japan SLC, Shizuoka, Japan) aged 6–7 weeks were housed at 24 ± 1 °C and 55 ± 10% humidity under a 12 h light/dark cycle with *ad libitum* access to water and MF chow (Oriental Yeast, Tokyo, Japan). The experimental protocols used in this study were approved by the Kyoto University Animal Research Committee (permit number 13–38) and performed in accordance with their ethical guidelines.

2.1.2. Experimental design

To investigate the acute effects of vasodilators, mice received intraperitoneal (i.p.) injections of L-OHP (10 mg/kg body weight [Wako Pure Chemical Industries, Osaka, Japan]) or vehicle (5% glucose solution) once a week for 4–6 weeks. Skin blood flow and cold and mechanical sensitivities (see below) were measured before and after the following drugs were administered: tadalafil (10 mg/kg [Combi-Blocks, San Diego, CA], dissolved in 10% DMSO and 20% polyethylene glycol 400 in distilled water), i.p., 1 h before testing; limaprost alfadex (0.3 mg/kg [Ono Pharmaceutical Co., Osaka, Japan] in saline) *per os*, 0.5 h before testing; bosentan (30 mg/kg [Toronto Research Chemicals, Toronto, Canada] in saline), i.p., 4 h before testing; vehicle, i.p., 1 h before testing.

To investigate the preventive effects of tadalafil, mice receiving i.p. injections of L-OHP (10 mg/kg) or vehicle once a week for 8 weeks were fed chow containing 0.1% (w/w) tadalafil (Oriental Yeast) *ad libitum* from the day before starting to the end of repeated injections (8 weeks). Skin blood flow and temperature, cold, mechanical, and current stimulation sensitivities, and nerve conduction velocities (NCVs) were measured after 8 weeks (see below).

2.1.3. Physiological analyses

Skin blood flow through the plantar surface of the hindpaw was measured using a small laser Doppler blood flow sensor (RBF-101; Pioneer, Tokyo, Japan), and the temperature was measured with a skin surface probe connected to a thermometer (BAT-10 multipurpose thermometer; Physitemp Instruments, Clifton NJ). Sensory and motor NCVs in the tail were recorded with an amplifier (EX-1; AD Instruments, Milford, MA) in response to electrical stimulation (STG4002; Multi Channel Systems, Reutlingen, Germany) and processed with LabChart8 software (AD Instruments).

2.1.4. Behavioral tests

Cold sensitivity was assessed by quantifying cold-escape behaviors for 60 s after acetone (10 μl) was applied to the plantar skin of the hind paw. Mechanical sensitivity was assessed by measuring the paw withdrawal threshold against stimulation with von Frey filaments (Stoelting, Wood Dale, IL). Thermal sensitivity was assessed by measuring the time to withdraw a paw exposed to a radiant heat source from a Hargreaves apparatus (Ugo Basile, Milan, Italy). Paw withdrawal thresholds to transcutaneous current stimuli were measured using a Neurometer CPT/C (Neurotron Inc., Baltimore, MD).

2.1.5. Immunohistochemistry

Immunohistochemistry for CD31, a platelet-endothelial cell adhesion molecule highly expressed in the vascular endothelial cells, was performed with 6 μm-thick sciatic nerves sections incubated with CD31 antibody (1:100; BD Bioscience, Franklin Lakes, NJ). The density of $CD31^+$ vessels (count/mm^2) was calculated.

2.1.6. Electron microscopy

Sciatic nerves fixed in 2% glutaraldehyde and 4% paraformaldehyde and postfixed in 1% osmium tetroxide were sectioned (70 nm) and imaged with a transmission electron microscope (H7650; Hitachi, Tokyo, Japan). The axons were classified by calculating the circularity and analyzed with MetaMorph software.

2.1.7. Platinum concentration

The concentrations of platinum in blood, sciatic nerve, and dorsal root ganglia (DRG) samples were measured with an Agilent 7700x ICP-MS system (Agilent Technologies, Santa Clara, CA).

2.1.8. Cytotoxicity assay

The cytotoxicity of L-OHP to human colon cancer (HCT116) and stomach adenocarcinoma (AGS) cell lines (ATCC, Manassas, VA) was assessed with an MTT assay.

2.1.9. Statistical analysis

Differences were compared using Student's *t* tests for two groups and one-way or two-way analyses of variance (ANOVA) followed by an appropriate *post hoc* test for more than two groups. Statistical analyses of the 50% withdrawal thresholds in von Frey filament testing were performed using Mann–Whitney *U* test or Kruskal–Wallis test followed by Dunn's *post hoc* test for each day. Other time-course data were analyzed by two-way ANOVA for repeated measures followed by Tukey's *post hoc* test. In all cases, differences of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Acute alleviative effects of vasodilators on L-OHP-induced peripheral vascular impairment and cold hypersensitivity

Skin blood flow through the hindpaw was measured before and after various vasodilators were administered to mice treated with L-OHP or vehicle for 4 weeks (Fig. 1A). Skin blood flow was significantly reduced by repeated injections of L-OHP. A single administration of the PDE5 inhibitor tadalafil (10 mg/kg), the

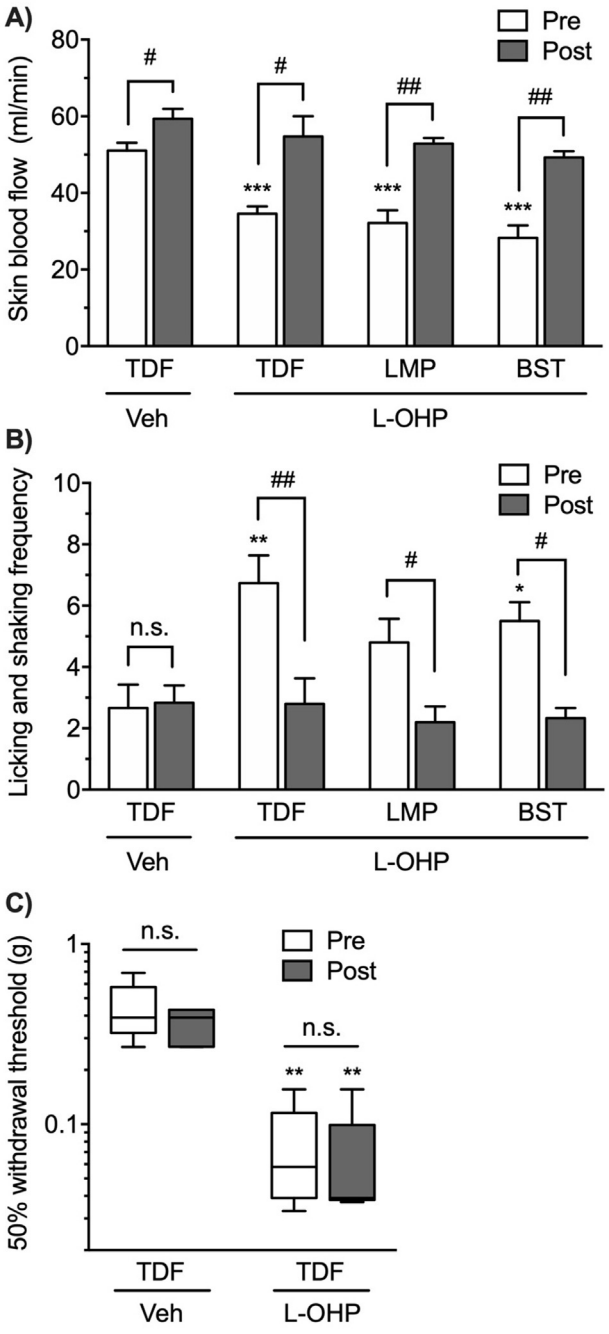

Fig. 1. *Acute effects of vasodilators on L-OHP-induced peripheral vascular impairment and peripheral neuropathy.* Oxaliplatin (L-OHP; 10 mg/kg) or its vehicle (Veh) was injected intraperitoneally once a week for 4–6 weeks. Skin blood flow in the hind paw (A) and cold sensitivity (B) were assessed before (pre) and after (post) administration of tadalafil (TDF; 10 mg/kg, i.p., 1 h before testing), limaprost alfadex (LMP; 0.3 mg/kg, *per os*, 0.5 h before testing), or bosentan (BST; 30 mg/kg, i.p., 4 h before testing) 4 and 5 weeks after the repeated injections, respectively. Skin blood flow (ml/min) in the hind paw was measured by using a small laser Doppler blood flow sensor. The frequency of cold-escape behaviors (licking and shaking) were measured for 60 s in response to an application of acetone. The results are expressed as means ± S.E.M. (n = 4–6). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the pre-values of vehicle-treated mice (unpaired t test); $\#P < 0.05$, $\#\#P < 0.01$ (paired t test); n.s., not significant. (C) The 50% withdrawal thresholds to mechanical stimulation with von Frey filaments were measured before (pre) and 1 h after (post) an administration of tadalafil (TDF; 10 mg/kg, i.p.) after 6 weeks of repeated injections of vehicle or L-OHP (n = 5–6). $^{**}P < 0.01$ compared with the pre values of vehicle-treated mice (Mann–Whitney U-test).

prostaglandin E_1 analog limaprost alfadex (0.3 mg/kg), and the ET receptor antagonist bosentan (30 mg/kg) significantly recovered the decreased skin blood flow in L-OHP-treated mice. A single administration of tadalafil also significantly increased the skin blood flow in the vehicle-treated mice.

The frequency of licking and shaking in response to an acetone application was increased in mice treated with L-OHP for 5 weeks. The cold hypersensitivity was significantly attenuated by a single administration of tadalafil, limaprost alfadex, and bosentan (Fig. 1B). The 50% withdrawal threshold to von Frey filaments were decreased in mice treated with L-OHP for 6 weeks. However, the mechanical hypersensitivity was not affected by a single administration of tadalafil (Fig. 1C).

3.2. Long-term administration of tadalafil improves peripheral vascular impairment in L-OHP-treated mice

We selected tadalafil for subsequent experiments because of its relatively long half-life and vasodilatory effects among the tested vasodilator drugs. We first verified that oral long-term administration of tadalafil did not affect the blood concentration and tissue accumulation of L-OHP in mice. The concentrations of platinum in blood and tissue samples, sciatic nerves and DRG, from mice fed a chow for 8 weeks containing 0.1% [w/w] tadalafil did not differ from those on the control (normal) diet (Table 1).

Repeated injections of L-OHP significantly reduced body weight. Long-term administration of tadalafil did not influence the body weight in repeated vehicle- and L-OHP-treated mice (Fig. 2A). Repeated L-OHP injections gradually and significantly reduced skin blood flow through the hindpaw. Significant reductions were observed between 4 and 8 weeks after the L-OHP injections. Tadalafil tended to increase the skin blood flow in the repeated vehicle-treated mice, and it significantly prevented the reduction in the repeated L-OHP-treated mice (Fig. 2B). Accordingly, skin temperature in the hindpaw was significantly lowered 8 weeks after the repeated L-OHP injections. The reduction of skin temperature was significantly prevented by tadalafil (Fig. 2C).

3.3. Long-term tadalafil prevents L-OHP-induced cold, mechanical, and current hypersensitivities and thermal hypoesthesia in L-OHP-treated mice

Repeated injections of L-OHP significantly increased the frequency of licking and shaking in response to acetone application, which developed within 2 weeks. The L-OHP-induced cold hypersensitivity was significantly prevented by long-term administration of tadalafil (Fig. 3A). Similarly, repeated L-OHP injections significantly decreased the 50% withdrawal threshold to mechanical stimulation between 2 and 8 week. The mechanical

Table 1
Effect of tadalafil on platinum concentrations in L-OHP-treated mice.

Sample type	Platinum concentration (nmol/mL or nmol/g)	
	L-OHP	L-OHP + TDF
Blood plasma	0.85 ± 0.15	0.92 ± 0.11
Sciatic nerves	4.21 ± 0.72	5.66 ± 1.31
DRG	5.55 ± 0.69	6.45 ± 0.15

L-OHP (10 mg/kg) was injected i.p. once a week for 8 weeks, during which mice were fed normal chow or a chow diet containing 0.1% tadalafil (TDF). Blood samples, sciatic nerves, and dorsal root ganglia (DRG) at L4–L6 were isolated from each group, and platinum concentrations were analyzed using an ICP-MS system. The results are expressed as means ± S.E.M (n = 4–5).

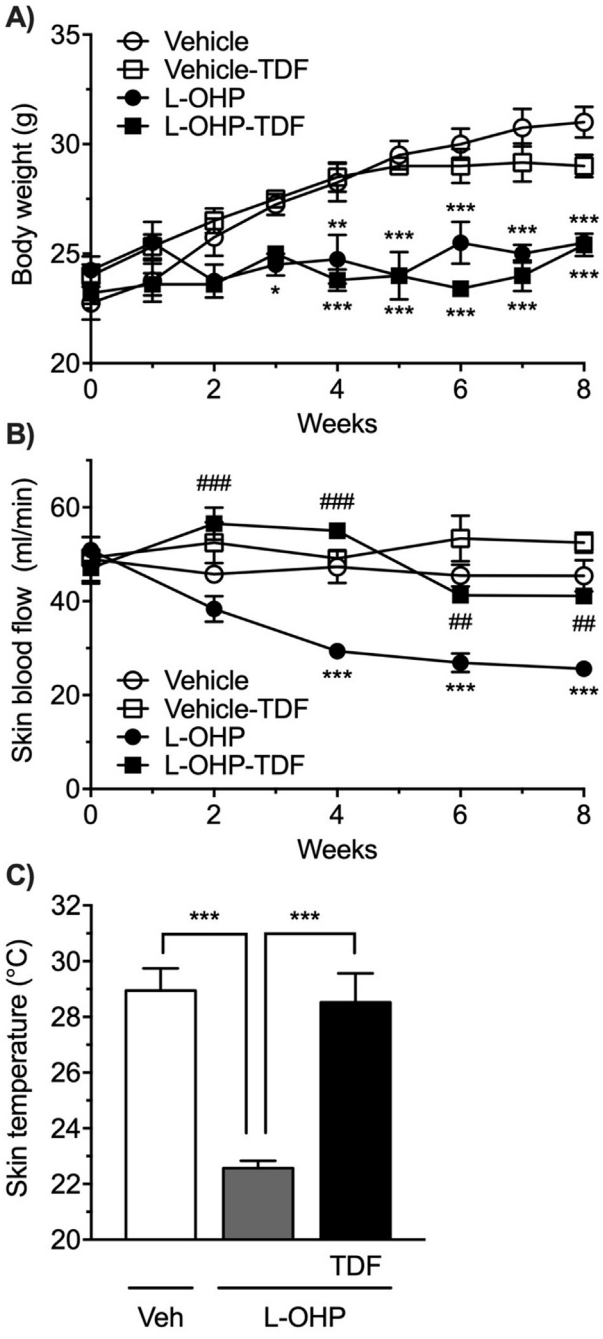

Fig. 2. *Effects of tadalafil and L-OHP on body weight and peripheral vascular impairment.* L-OHP (10 mg/kg) or its vehicle (Veh) was injected i.p. once a week for 8 weeks, during which mice were fed normal chow or a chow diet containing 0.1% tadalafil (TDF). Body weight (A) and skin blood flow through the hindpaw (B) were measured before and weekly or biweekly ($n = 4$–6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the vehicle-treated mice without tadalafil; $\#\#P < 0.01$, $\#\#\#P < 0.001$ compared with the L-OHP-treated mice without tadalafil (Tukey's *post hoc* test following two-way repeated measured ANOVA). (C) Skin temperature in the hind paw was measured after 8 weeks of repeated injections ($n = 8$). $^{***}P < 0.001$ (Tukey's *post hoc* test following one-way ANOVA). The results are expressed as means ± S.E.M.

hypersensitivity was significantly attenuated by long-term tadalafil administration (Fig. 3B). By contrast, repeated injections of L-OHP for 8 weeks significantly prolonged the latency of thermal nociceptive responses. The thermal hypoesthesia, a symptom of cumulative peripheral neuropathy,[22] was significantly prevented by long-term tadalafil administration (Fig. 3C).

We measured current perception thresholds to sine-wave pulses of 5, 250, and 2000 Hz produced by a Neurometer to stimulate C, Aδ, and Aβ fibers, respectively. Current perception thresholds at these frequencies were significantly decreased by repeated L-OHP injections for 8 weeks. Long-term tadalafil administration prevented these decreases at any frequencies (Fig. 3D–F).

3.4. Long-term tadalafil preserves sensory NCV in L-OHP-treated mice

We measured sensory and motor NCVs in the tails of mice receiving repeated L-OHP injections for 8 weeks. Sensory NCV was significantly reduced in mice treated with L-OHP. Long-term tadalafil administration prevented the decrease in sensory NCV (Fig. 4A). L-OHP treatment also reduced motor NCV, but this decrease and the effect of tadalafil were not significant (Fig. 4B).

3.5. Long-term tadalafil prevents L-OHP-induced decreases in endoneurial microvessel density in sciatic nerves

Immunostaining for CD-31, an endothelial marker, was performed on sciatic nerve sections from mice receiving repeated L-OHP injections for 8 weeks (Fig. 5A). Quantitative analysis of confocal images showed that the density of CD31-immunoreactive microvessels was significantly reduced by L-OHP, which was prevented by long-term administration of tadalafil (Fig. 5B).

3.6. Long-term tadalafil prevents L-OHP-induced axon degeneration in sciatic nerves

Electron microscopy of sciatic nerve sections revealed a degeneration of myelinated fibers with abnormal distorted morphology in mice receiving repeated L-OHP injections for 8 weeks (Fig. 6A). The percentage of axons with a circularity measure of >0.7 (indicative of normal axon morphology) was significantly reduced in L-OHP-treated mice. Consistently, the percentage of axons with a circularity of <0.5 (indicative of severe axonal degeneration) was significantly increased in these mice. Long-term tadalafil administration tended to prevent the changes in the distribution of axon circularities (Fig. 6B).

3.7. Tadalafil does not affect the cytotoxicity of L-OHP in human cancer cells

To verify that tadalafil did not impact the cytotoxicity of L-OHP on cancer cells, we performed *in vitro* MTT assays. The viability of HCT116 and AGS cells was not altered by exposure to various concentrations of tadalafil (up to 10 μM) for 24 h. Furthermore, tadalafil did not alter the reduced viability of cell lines exposed to 10 μM L-OHP for 24 h (Fig. 7). Thus, the above-described effects of tadalafil on L-OHP-induced peripheral neuropathy did not interfere with the ability of L-OHP to kill cancer cells.

4. Discussion

In the present study, we provided evidences that improvement of peripheral vascular impairment by tadalafil, a PDE5 inhibitor, showed acute alleviative and preventive effects on OIPN. A single administration of tadalafil exerted limited efficacy on cold hypersensitivity, but not on mechanical hypersensitivity. However, long-term administration of tadalafil had wide range effects on signs, symptoms and pathophysiological changes observed in OIPN model mice.

Patients treated with cumulative doses of L-OHP experience multiple sensory disturbances; cold hypersensitivity, but

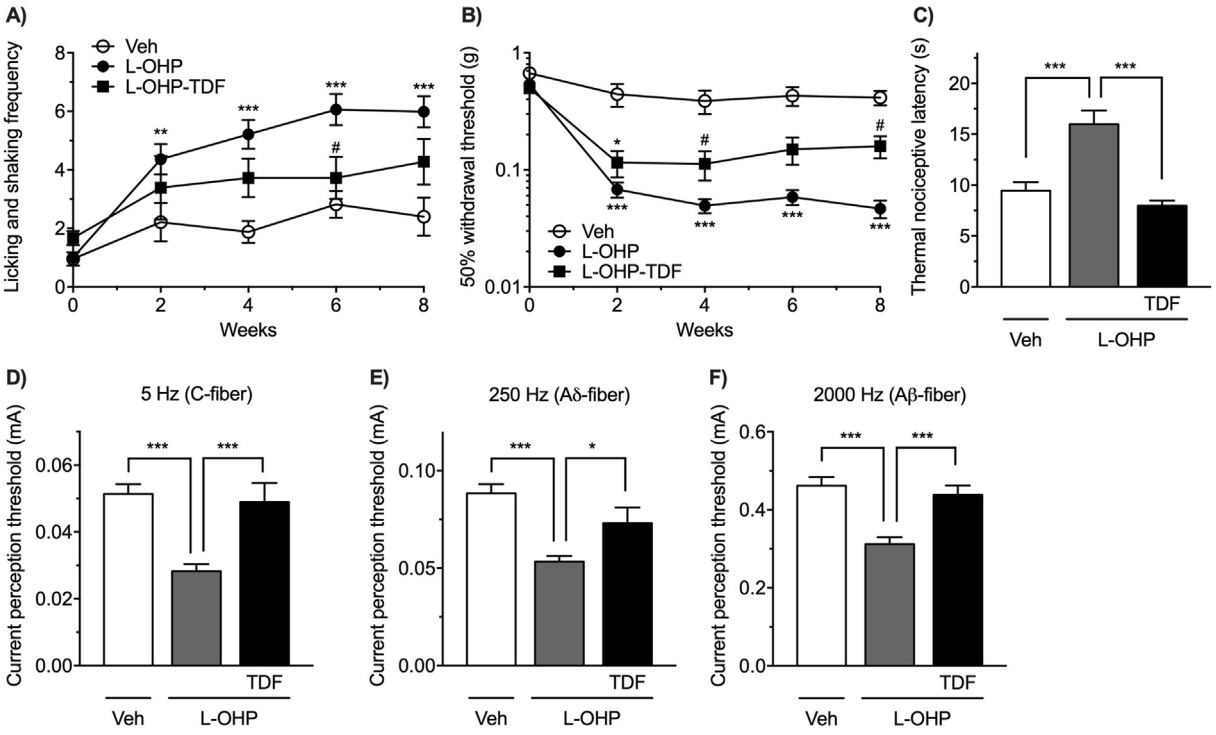

Fig. 3. *Effects of tadalafil on L-OHP-induced cold, mechanical, and current hypersensitivities and thermal hypoesthesia.* L-OHP (10 mg/kg) or its vehicle (Veh) was injected i.p. once a week for 8 weeks, during which mice were fed normal chow or a chow diet containing 0.1% tadalafil (TDF). Cold (A, $n = 6–11$) and mechanical (B, $n = 5–12$) sensitivities were assessed by acetone and von Frey filament tests, respectively, before injections and biweekly after. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the vehicle-treated mice without tadalafil; $\#P < 0.05$ compared with the L-OHP-treated mice without tadalafil (A, Tukey's *post hoc* test following two-way repeated measured ANOVA; B, Dunn's post *hoc test* following Kruskal–Wallis test). (C) Thermal sensitivity was assessed by Hargreaves test after 8 weeks of repeated injections ($n = 5–8$). $^{***}P < 0.001$ (Tukey's *post hoc* test following one-way ANOVA). (D–F) The current perception thresholds (mA) eliciting paw withdrawal responses to the sine-wave pulses at 5 Hz (A; C fiber), 250 Hz (B; Aδ fiber), and 2000 Hz (C; Aβ fiber) were measured after 8 weeks of repeated injections ($n = 8–13$). $^{*}P < 0.05$, $^{***}P < 0.001$ (Tukey's *post hoc* test following one-way ANOVA). The results are expressed as means ± S.E.M.

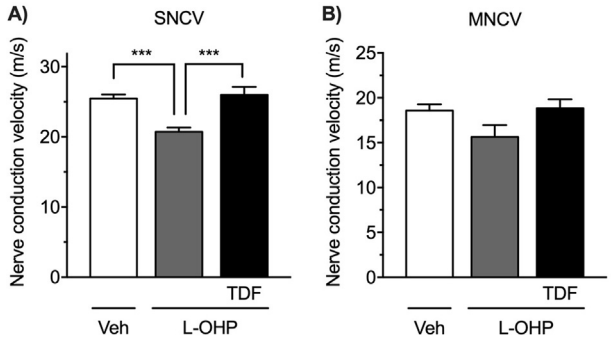

Fig. 4. *Effect of tadalafil on L-OHP-induced decreases in nerve conduction.* L-OHP (10 mg/kg) or its vehicle (Veh) was injected i.p. once a week for 8 weeks, during which mice were fed normal chow or a chow diet containing 0.1% tadalafil (TDF). Sensory (A) and motor (B) NCVs were measured in the mouse tails ($n = 8–13$). $^{***}P < 0.001$ (Tukey's *post hoc* test following one-way ANOVA). The results are expressed as means ± S.E.M.

concomitantly also hypoesthesia in warm, cool, touch and bump detection,[23] accompanied by a reduction of NCV.[24] However, there are no reliable animal models of OIPN, in which such sensory disturbances can be appropriately assessed. Mechanical and cold hypersensitivities have been frequently documented in animals receiving relatively low-doses of L-OHP (2–5 mg/kg) once or several times a week.[22,25–27] However, mice treated with these doses in our preliminary experiments did not exhibit thermal hypoesthesia, reduced NCV, or axonal degeneration (data not shown). In this study, we selected relatively higher-dose of L-OHP (10 mg/kg) once a week for 4–8 weeks. It enabled us to examine the effects of test drugs on abnormal sensations (mechanical/cold/current hypersensitivity and thermal hypoesthesia), neurological dysfunction and axon degeneration, although it caused the decrease in body weight.

Consistent with the previous experiments in animals[21,22] and patients,[23] peripheral blood flow was gradually reduced along with increasing cumulative doses of L-OHP, and it was accompanied with the reduction in skin temperature. Although the mechanisms underlying the peripheral vascular impairment are largely unknown, L-OHP can directly and/or indirectly affect vascular endothelial function,[28] and L-OHP-based chemotherapy induces vascular endothelial injury in colorectal cancer patients.[29,30] It is possible that the vascular endothelial injury and dysfunction induced by L-OHP may lead to the peripheral vascular impairment.

In the present study, mechanical hypersensitivity was invariably observed during the observation period. By contrast, cold hypersensitivity was gradually exacerbated along with increasing cumulative doses of L-OHP, which was likely paralleled by the progression of peripheral vascular impairment. L-OHP can induce rapid-onset cold hypersensitivity, a characteristic symptom of acute OIPN, within several hours after the injection.[26,27,31] We and other groups previously found that it is mediated through an L-OHP metabolite, oxalate,[27,31] which indirectly sensitizes a redox-sensitive nociceptor, transient receptor potential ankyrin 1 (TRPA1), against cold temperature.[32,33] However, as the cold hypersensitivity usually improves within a week, cumulative doses of L-OHP escalate the cold hypersensitivity through other mechanisms. In this study, three different vasodilators, namely, tadalafil, limaprost alfadex, and bosentan, attenuated the cold hypersensitivity while improving skin blood flow. These findings suggest that

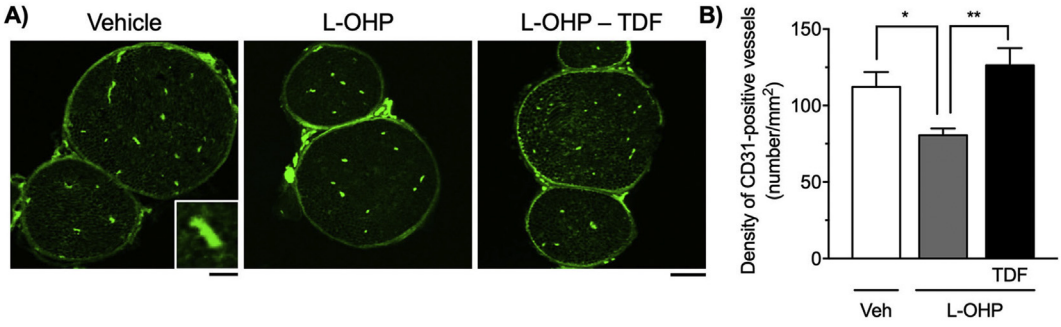

Fig. 5. *Effect of tadalafil on L-OHP-induced decrease in the density of endoneurial microvessels in the sciatic nerve.* L-OHP (10 mg/kg) or its vehicle (Veh) was injected i.p. once a week for 8 weeks, during which mice were fed normal chow or a chow diet containing 0.1% tadalafil (TDF). (A) Representative confocal fluorescence photographs of CD31-immunoreactive staining of endoneurial microvessels in cross-sections of sciatic nerves. Scale bar = 100 μm (20 μm in an enlarged image). (B) Quantitative analysis of the density of CD31-immunoreactive vessels (number/mm^2) (n = 5–11). *P < 0.05, **P < 0.01 (Tukey's *post hoc* test following one-way ANOVA). The results are expressed as means ± S.E.M.

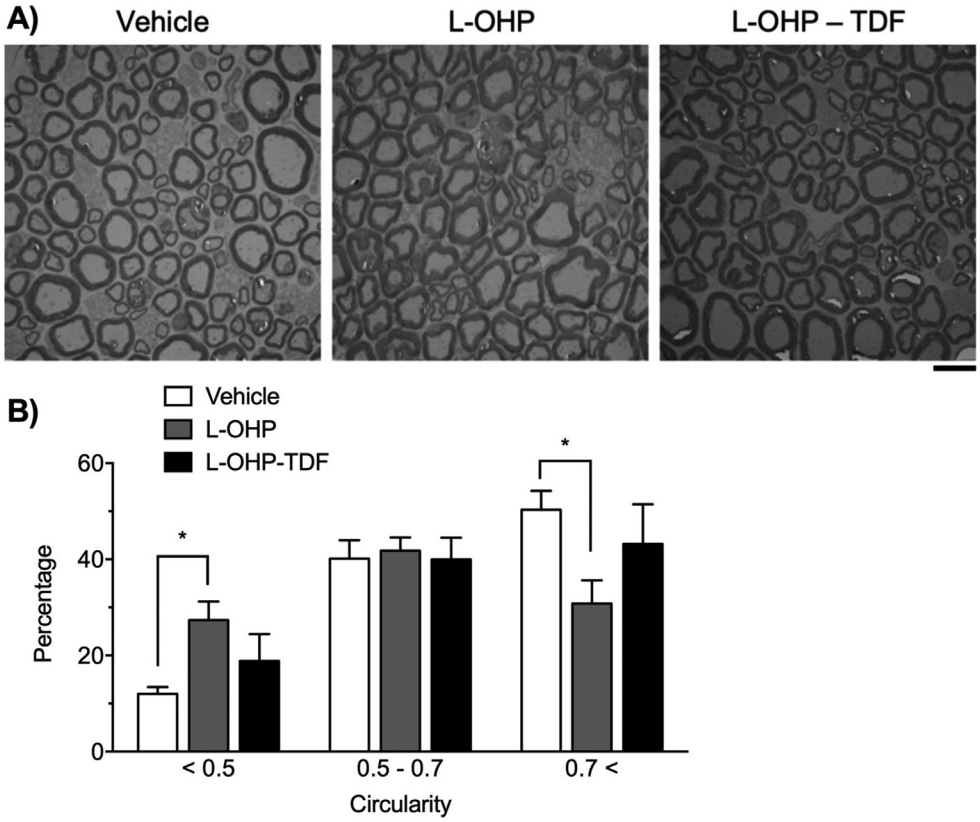

Fig. 6. *Effect of tadalafil on L-OHP-induced axonal degeneration in the sciatic nerve.* L-OHP (10 mg/kg) or its vehicle was injected i.p. once a week for 8 weeks, during which mice were fed normal chow or a chow diet containing 0.1% tadalafil (TDF). (A) Representative electron light micrographs of cross-sections of the sciatic nerves from vehicle- and L-OHP-treated mice with or without tadalafil. Scale bar = 10 μm. (B) Quantitative analysis of the distribution of axon circularity in the sciatic nerves. The percentages of the myelinated fibers showing axon circularity >0.7, between 0.5 and 0.7, and <0.5 are presented (n = 7–10). *P < 0.05 (Tukey's *post hoc* test following one-way ANOVA). The results are expressed as means ± S.E.M.

cold hypersensitivity can be mitigated by an increase in peripheral blood flow. We recently showed that hypoxia resulting from peripheral vascular impairment sensitizes TRPA1 in diabetic peripheral neuropathy and hindlimb ischemic mouse models.[15,34] Thus, it is conceivable that the acute effect of vasodilators on cold hypersensitivity may be mediated by a suppression of hypoxia-induced TRPA1 sensitization. By contrast, a single administration of tadalafil had no effect on the L-OHP-induced mechanical hypersensitivity, consistent with our previous findings in diabetic neuropathy and hindlimb ischemic mouse models.[34]

The key finding of this study is that the progressive improvements of peripheral vascular impairment and rewarming by tadalafil attenuated a variety of symptoms associated with OIPN, including mechanical, cold, and current hypersensitivities, thermal hypoesthesia, decreased sensory NCV and microvessel density, and axon degeneration in the sciatic nerves. These inhibitory effects are unlikely to be caused by the altered blood concentration and accumulation of L-OHP in peripheral neurons. Mechanical, cold, and current hypersensitivities are generally considered to be mediated through the sensitization of nociceptors and enhanced excitability of nociceptive primary sensory neurons (peripheral sensitization), as well as synaptic facilitation and enhanced responsiveness of nociceptive dorsal horn neurons (central sensitization). Peripheral and central sensitization in OIPN are

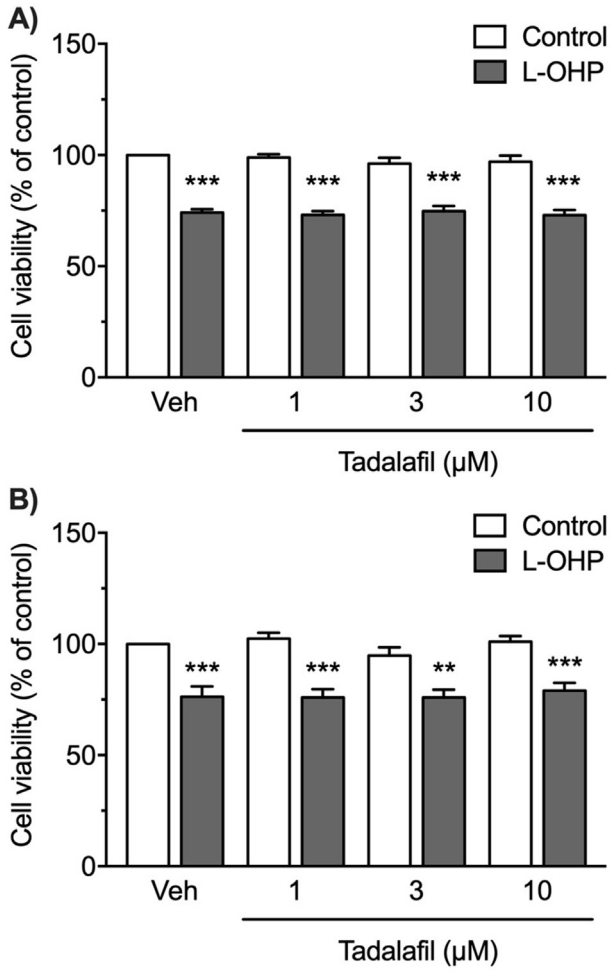

Fig. 7. *Effect of tadalafil on L-OHP-induced cytotoxicity in cultured human cancer cell lines.* HCT116 (A) and AGS (B) human cancer cell lines were incubated with vehicle (0.1% DMSO; control) or L-OHP (10 μM) for 24 h in the presence or absence of tadalafil (TDF; 1–10 μM). Then, the cell viability was measured by an MTT assay. The results are expressed as percentages relative to control cells ($n = 4–5$). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control cells (Tukey's post *hoc test* following two-way ANOVA). The results are expressed as means ± S.E.M.

considered to be due to alterations of voltage-gated ion channels and TRP channels, dysregulation of calcium homeostasis, spinal glial activation and so on.[1,3] However, taken together with the findings that mechanical hypersensitivity was not affected by transient vasodilatory effect of tadalafil, it is conceivable that continuous preservation of peripheral blood flow might prevent the induction and/or progression of OIPN, resulting prevention of peripheral and central sensitization, rather than inhibition of these enhanced pain signaling. Pain in OIPN can be inhibited by analgesics or drugs available for neuropathic pain.[35] In this context, the guideline for chemotherapy-induced peripheral neuropathy moderately recommends a serotonin noradrenalin reuptake inhibitor duloxetine, and may offer to try tricyclic antidepressants, or gabapentin/pregabalin, while it also describes that these drugs have no effect on hypoesthesia and no preventive effect on OIPN.[35] Our findings further suggest that long-term administration of tadalafil prevents OIPN, rather than simply providing analgesic effects.

Several lines of evidence suggest that impaired endoneurial microvascular function and the resultant perineural hypoxia and malnutrition contribute to the pathogenesis of peripheral neuropathy.[5,6] Perineural hypoxia caused by decreased nerve blood flow alters nerve function, and exacerbates nerve degeneration through production of oxidative stress.[36] Moreover, increased oxidative stress causes further endoneurial microvascular dysfunction.[6,37] Notably, oxidative stress from mitochondrial dysfunction is a major cause of L-OHP-induced neurotoxicity,[38] and antioxidants can reduce OIPN.[39] In this way, endoneurial microvascular dysfunction may contribute to the progression of L-OHP-induced neurotoxicity. Our present data showed that tadalafil administration inhibited the reduced number of CD31-immunoreactive microvessels in the sciatic nerves, suggesting that tadalafil can preserve L-OHP-induced endoneurial microvascular dysfunction. This conservation of the endoneurial environment and thus prevention of perineural hypoxia and malnutrition may contribute to the preservation of axon morphology in sciatic nerves. It may be interesting to determine whether tadalafil administration can reduce the level of oxidative stress in OIPN model mice. However, we could not fully exclude other mechanisms, such as direct protective effects of tadalafil on primary sensory neurons and/or surrounding myelinated Schwann cells. Further investigations are needed to identify the underlying mechanisms.

By contrast, we and other groups recently reported that reduction of peripheral blood flow by cooling or compressing the extremities with frozen or surgical gloves can suppress taxanes-induced peripheral neuropathy in breast cancer patients.[40,41] The cryotherapy or compression therapy seemingly contradicts the present results. However, they are performed to prevent the distribution of the anti-cancer agent into the extremities only just before, during and after the intravenous infusion. When the blood concentration of L-OHP is high, intake of vasodilators is likely to increase the distribution into the peripheral area, and to possibly exacerbate OIPN. Thus, it may be better to prevent OIPN by stopping vasodilators before, during and after L-OHP injection, but by recovering peripheral blood flow when the blood concentration of L-OHP is low.

In conclusion, the results from the present studies show that the improvement of peripheral vascular impairment by tadalafil, a PDE5 inhibitor, alleviates cold hypersensitivity and prevents the progression of OIPN, resulting in inhibition of abnormal sensations, neurological dysfunction and morphological neurodegeneration. Furthermore, we confirmed that tadalafil do not impact the antitumor effect of L-OHP *in vitro*. Although it will be needed to determine whether continuous increase in blood flow affects tumor growth and antitumor effect of L-OHP in tumor-bearing animal experiments, our data suggest that vasodilators, such as tadalafil, are potential therapeutic and preventive options for OIPN.

Declaration of Competing Interest

The authors declare no potential conflicts of interest associated with this manuscript.

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References

1. Colvin LA. Chemotherapy-induced peripheral neuropathy: where are we now? *Pain*. 2019;160(Suppl 1):S1–S10. https://doi.org/10.1097/j.pain.0000000000001540.
2. Tabata A, Kanai M, Horimatsu T, et al. Changes in upper extremity function, ADL, and HRQoL in colorectal cancer patients after the first chemotherapy cycle with oxaliplatin: a prospective single-center observational study. *Support Care Cancer*. 2018;26:2397–2405. https://doi.org/10.1007/s00520-018-4070-z.
3. Flatters SJL, Dougherty PM, Colvin LA. Clinical and preclinical perspectives on Chemotherapy-Induced Peripheral Neuropathy (CIPN): a narrative review. *Br J Anaesth*. 2017;119:737–749. https://doi.org/10.1093/bja/aex229.
4. Hu LY, Mi WL, Wu GC, et al. Prevention and treatment for chemotherapy-induced peripheral neuropathy: therapies based on CIPN mechanisms. *Curr Neuropharmacol*. 2019;17:184–196. https://doi.org/10.2174/1570159X15666170915143217.
5. Nukada H. Ischemia and diabetic neuropathy. *Handb Clin Neurol*. 2014;126:469–487. https://doi.org/10.1016/B978-0-444-53480-4.00023-0.
6. Yorek MA. Vascular impairment of epineurial arterioles of the sciatic nerve: implications for diabetic peripheral neuropathy. *Rev Diabet Stud*. 2015;12:13–28. https://doi.org/10.1900/RDS.2015.12.13.
7. Quattrini C, Harris ND, Malik RA, et al. Impaired skin microvascular reactivity in painful diabetic neuropathy. *Diabetes Care*. 2007;30:655–659. https://doi.org/10.2337/dc06-2154.
8. Charles M, Soedamah-Muthu SS, Tesfaye S, et al. Low peripheral nerve conduction velocities and amplitudes are strongly related to diabetic microvascular complications in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetes Care*. 2010;33:2648–2653. https://doi.org/10.2337/dc10-0456.
9. Kambiz S, van Neck JW, Cosgun SG, et al. An early diagnostic tool for diabetic peripheral neuropathy in rats. *PLoS One*. 2015;10, e0126892. https://doi.org/10.1371/journal.pone.0126892.
10. Rüger LJ, Irnich D, Abahji TN, et al. Characteristics of chronic ischemic pain in patients with peripheral arterial disease. *Pain*. 2008;139:201–208. https://doi.org/10.1016/j.pain.2008.03.027.
11. Devulder J, van Suijlekom H, van Dongen R, et al. 25. Ischemic pain in the extremities and Raynaud's phenomenon. *Pain Pract*. 2011;11:483–491. https://doi.org/10.1111/j.1533-2500.2011.00460.x.
12. Flavahan NA. A vascular mechanistic approach to understanding Raynaud phenomenon. *Nat Rev Rheumatol*. 2015;11:146–158. https://doi.org/10.1038/nrrheum.2014.195.
13. Hao JX, Blakeman KH, Yu W, et al. Development of a mouse model of neuropathic pain following photochemically induced ischemia in the sciatic nerve. *Exp Neurol*. 2000;163:231–238. https://doi.org/10.1006/exnr.2000.7373.
14. Coderre TJ, Xanthos DN, Francis L, et al. Chronic post-ischemia pain (CPIP): a novel animal model of complex regional pain syndrome-Type I (CRPS-I; reflex sympathetic dystrophy) produced by prolonged hindpaw ischemia and reperfusion in the rat. *Pain*. 2004;112:94–105. https://doi.org/10.1016/j.pain.2004.08.001.
15. So K, Tei Y, Zhao M, et al. Hypoxia-induced sensitization of TRPA1 in painful dysesthesia evoked by transient hindlimb ischemia/reperfusion in mice. *Sci Rep*. 2016;6:23261. https://doi.org/10.1038/srep23261.
16. Kihara M, Schmelzer JD, Low PA. Effect of cilostazol on experimental diabetic neuropathy in the rat. *Diabetologia*. 1995;38:914–918.
17. Wang L, Chopp M, Szalad A, et al. Sildenafil ameliorates long term peripheral neuropathy in type II diabetic mice. *PLoS One*. 2015;10, e0118134. https://doi.org/10.1371/journal.pone.0118134.
18. Wang L, Chopp M, Szalad A, et al. Tadalafil promotes the recovery of peripheral neuropathy in type II diabetic mice. *PLoS One*. 2016;11, e0159665. https://doi.org/10.1371/journal.pone.0159665.
19. Sonobe M, Yasuda H, Hisanaga T, et al. Amelioration of nerve Na^+-K^+-ATPase activity independently of myo-inositol level by PGE_1 analogue OP-1206.alpha-CD in streptozocin-induced diabetic rats. *Diabetes*. 1991;40:726–730. https://doi.org/10.2337/diab.40.6.726.
20. Cameron NE, Dines KC, Cotter MA. The potential contribution of endothelin-1 to neurovascular abnormalities in streptozotocin-diabetic rats. *Diabetologia*. 1994;37:1209–1215.
21. Gauchan P, Andoh T, Kato A, et al. Effects of the prostaglandin E_1 analog limaprost on mechanical allodynia caused by chemotherapeutic agents in mice. *J Pharmacol Sci*. 2009;109:469–472.
22. Kono T, Suzuki Y, Mizuno K, et al. Preventive effect of oral goshajinkigan on chronic oxaliplatin-induced hypoesthesia in rats. *Sci Rep*. 2015;5:16078. https://doi.org/10.1038/srep16078.
23. de Carvalho Barbosa M, Kosturakis AK, Eng C, et al. A quantitative sensory analysis of peripheral neuropathy in colorectal cancer and its exacerbation by oxaliplatin chemotherapy. *Cancer Res*. 2014;74:5955–5962. https://doi.org/10.1158/0008-5472.CAN-14-2060.
24. Matsuoka A, Mitsuma A, Maeda O, et al. Quantitative assessment of chemotherapy-induced peripheral neurotoxicity using a point-of-care nerve conduction device. *Cancer Sci*. 2016;107:1453–1457. https://doi.org/10.1111/cas.13010.
25. Cavaletti G, Tredici G, Petruccioli MG, et al. Effects of different schedules of oxaliplatin treatment on the peripheral nervous system of the rat. *Eur J Cancer*. 2001;37:2457–2463.
26. Ling B, Coudoré-Civiale MA, Balayssac D, et al. Behavioral and immunohistological assessment of painful neuropathy induced by a single oxaliplatin injection in the rat. *Toxicology*. 2007;234:176–184. https://doi.org/10.1016/j.tox.2007.02.013.
27. Sakurai M, Egashira N, Kawashiri T, et al. Oxaliplatin induced neuropathy in the rat: involvement of oxalate in cold hyperalgesia but not mechanical allodynia. *Pain*. 2009;147:165–174. https://doi.org/10.1016/j.pain.2009.09.003.
28. Pan Y, Chen F, Huang S, et al. TRPA1 and TRPM8 receptors may promote local vasodilation that aggravates oxaliplatin-induced peripheral neuropathy amenable to 17β-estradiol treatment. *Curr Neurovascular Res*. 2016;13:309–317.
29. Jensen SA, Sørensen JB. 5-Fluorouracil-based therapy induces endovascular injury having potential significance to development of clinically overt cardiotoxicity. *Cancer Chemother Pharmacol*. 2012;69:57–64. https://doi.org/10.1007/s00280-011-1669-x.
30. Robinson SM, Mann J, Vasilaki A, et al. Pathogenesis of FOLFOX induced sinusoidal obstruction syndrome in a murine chemotherapy model. *J Hepatol*. 2013;59:318–326. https://doi.org/10.1016/j.jhep.2013.04.014.
31. Zhao M, Isami K, Nakamura S, et al. Acute cold hypersensitivity characteristically induced by oxaliplatin is caused by the enhanced responsiveness of TRPA1 in mice. *Mol Pain*. 2012;8:55. https://doi.org/10.1186/1744-8069-8-55.
32. Miyake T, Nakamura S, Zhao M, et al. Cold sensitivity of TRPA1 is unveiled by the prolyl hydroxylation blockade-induced sensitization to ROS. *Nat Commun*. 2016;7:12840. https://doi.org/10.1038/ncomms12840.
33. Miyake T, Nakamura S, Zhao M, et al. Distinct mechanism of cysteine oxidation-dependent activation and cold sensitization of human transient receptor potential ankyrin 1 channel by high and low oxaliplatin. *Front Physiol*. 2017;8:878. https://doi.org/10.3389/fphys.2017.00878.
34. Hiyama H, Yano Y, So K, et al. TRPA1 sensitization during diabetic vascular impairment contributes to cold hypersensitivity in a mouse model of painful diabetic peripheral neuropathy. *Mol Pain*. 2018;14. https://doi.org/10.1177/1744806918789812, 1744806918789812.
35. Hershman DL, Lacchetti C, Dworkin RH, et al. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. *J Clin Oncol*. 2014;32:1941–1967. https://doi.org/10.1200/JCO.2013.54.0914.
36. Coppey LJ, Davidson EP, Dunlap JA, et al. Slowing of motor nerve conduction velocity in streptozotocin-induced diabetic rats is preceded by impaired vasodilation in arterioles that overlie the sciatic nerve. *Int J Exp Diabetes Res*. 2000;1:131–143.
37. Kelly BD, Hackett SF, Hirota K, et al. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. *Circ Res*. 2003;93:1074–1081. https://doi.org/10.1161/01.RES.0000102937.50486.1B.
38. Trecarichi A, Flatters SJL. Mitochondrial dysfunction in the pathogenesis of chemotherapy-induced peripheral neuropathy. *Int Rev Neurobiol*. 2019;145:83–126. https://doi.org/10.1016/bs.irn.2019.05.001.
39. Carvalho LF, Silva AMF, Carvalho AA. The use of antioxidant agents for chemotherapy-induced peripheral neuropathy treatment in animal models. *Clin Exp Pharmacol Physiol*. 2017;44:971–979. https://doi.org/10.1111/1440-1681.12803.
40. Hanai A, Ishiguro H, Sozu T, et al. Effects of cryotherapy on objective and subjective symptoms of paclitaxel-induced neuropathy: prospective self-controlled trial. *J Natl Cancer Inst*. 2018;110:141–148. https://doi.org/10.1093/jnci/djx178.
41. Tsuyuki S, Senda N, Kanng Y, et al. Evaluation of the effect of compression therapy using surgical gloves on nanoparticle albumin-bound paclitaxel-induced peripheral neuropathy: a phase II multicenter study by the Kamigata Breast Cancer Study Group. *Breast Canc Res Treat*. 2016;160:61–67. https://doi.org/10.1007/s10549-016-3977-7.